UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT 29 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Additional Mutagenicity Data Resubmitted in Support of the Registration of Thiophanate Methyl (EPA Reg. No. 4581-322) Tox. Proj. No. 7-1019. Tox. Chem. No. 375A.

TO: Lois Rossi, Product Manager
Registration Division (TS-767C)

Thru: Judith Hauswirth, Ph. D., Acting Head
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS 769)

Judith W. Hauswirth 10/28/87

FROM: Roger Gardner, Toxicologist
Review Section 6
Toxicology Branch
Hazard Evaluation Division (TS-769)

Roger Gardner 10-29-87

10/31/87

Action Requested

Review of the following mutagenicity studies:

1. Tippins, R. S. September 28, 1984 Gene Mutation in Chinese Hamster V79 Cells -- Thiophanate-Methyl Technical. Unpublished report no. 063013-M-05184 prepared by Life Science Research, Rome Toxicology Centre. Submitted by Penwalt Corp. MRID No. 40005504

2. Myhr, B. C. October, 1981. Evaluation of Pure Thiophanate-Methyl in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay Unpublished report no. 21191. prepared by Litton Bionetics, Inc. Submitted by Pennwalt Corp. MRID No. 40095503

Recommendations and Conclusions

1. No mutagenic activity was observed in Chinese Hamster V79 cells exposed to 6.25 to 100 ug thiophanate methyl per ml with and without metabolic activation (see Appendix below).

2. Concentrations of 5 to 1000 ug thiophanate methyl per ml did not induce unscheduled DNA synthesis in primary rat hepatocytes in vitro (see Appendix below).

3. The new studies do not specifically evaluate potential effects of thiophanate methyl on chromosomal structure. Therefore, additional mutagenicity testing is still needed to satisfy the mutagenicity requirements.

3

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TABLE 1

SUMMARY OF DATA FROM RAT HEPATOCYTE UDS ASSAY

CLIENT: Nippon Soda LBI ASSAY NO. 5869 ASSAY INITIATION DATE: September 16, 1981

CLIENT'S CODE: Pure thiophanate-methyl Lot CP-10B SOLVENT: DMSO TECH: Marie McKeon

Test Condition	Concentration	UDS* grains/ nucleus	Avg.† % nuclei with ≥ 6 grains	Avg.† % nuclei with ≥ 20 grains	Survival†† 22 hrs, %
Solvent Control	1% DMSO	0.92	2.0	0.0	100.0
Positive Control (2-AAF)	0.05 µg/ml	18.06	97.3	43.3	96.9
Test Material					
[a]Thiophanate-methyl	1000 µg/ml	1.63	7.4	0.0	38.0
[a]Thiophanate-methyl	500 µg/ml	1.01	2.7	0.0	81.6
Thiophanate-methyl	250 µg/ml	0.72	0.7	0.0	93.5
Thiophanate-methyl	100 µg/ml	0.78	0.7	0.0	98.8
Thiophanate-methyl	50 µg/ml	0.61	0.0	0.0	102.9
Thiophanate-methyl	25 µg/ml	0.53	0.0	0.0	109.6
Thiophanate-methyl	10 µg/ml	0.63	0.7	0.0	ND
[a]Thiophanate-methyl	5 µg/ml	0.40	0.0	0.0	ND

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*UDS = Average of net nuclear grain counts on triplicate coverslips (150 total cells).
†Average values for triplicate coverslips.
††Survival = Number of viable cells per unit area relative to the solvent control x 100%.
2-AAF = 2-acetyl aminofluorene
[a]Values represent 75 cells on each of two coverslips (150 total cells); the third coverslip was lost to technical

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ADDENDUM

Reported Results from an Unscheduled DNA Synthesis
Assay with Thiophanate Methyl in Primary Rat Hepatocytes

MRID No. 40095503

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17

II. REPORTED RESULTS

The report noted that a concentration of 1000 ug thiophanate methyl per ml WME was insoluble. However, the test substance was soluble in DMSO at a concentration of 100,000 ug/ml after vigorous mixing. In diluting DMSO solutions of the test substance with WME to obtain test concentrations of 250 ug/ml and higher, the investigators noted a cloudiness immediately on mixing of the DMSO and culture medium, but the test substance appeared to redissolve at the 250 and 500 ug/ml concentrations. The 1000 ug/ml concentration was the only one that did not redissolve. During the survival analysis, the investigators noted that crystalline test material appeared on the monolayers of cultures exposed to 500 and 1000 ug/ml concentrations.

The report indicated that approximately 65% of the hepatocytes collected for the UDS assay were viable, and approximately 77% of those attached to the coverslips during the 1.5-hour attachment period. Ninety-eight per cent of the attached cells were reported to be viable. In addition, the investigators noted that the survival in the solvent control after incubation under test and control conditions was 106% of the cell count determined after the attachment period. According to the report, these results indicated that the cultures were in good metabolic condition for the UDS assay.

The results of the UDS assay are included as reported in the Addendum below. They did not indicate that the criteria for a positive result were met.

B. Methods (continued)

were developed, fixed and stained with Williams' modified hematoxylin and eosin procedure.

Nuclei were evaluated under 1500X magnification for UDS by counting the number of nuclear grains and subtracting the average number of grains in three nuclear-sized areas adjacent to each nucleus. The result is called the net grain count.

4. Evaluation criteria: The report described the criteria used as follows:

> The net nuclear grain count is determined for 50 randomly selected cells on each coverslip. Only normally-appearing nuclei are scored, and any occasional nuclei blackened by grains too numberous to count are excluded as cells in which replicative DNA synthesis occurred rather than repair synthesis. If the actual count for any nucleus is less than zero (i. e., cytoplasmic count is greater than nuclear count), a net value of zero is used in the calculation of a mean value. The mean net nuclear grain count is determined from the triplicate coverslips (150 nuclei total) for each treatment condition...
>
> The test material is considered to be active in the UDS assay at applied concentrations that cause:
>
> 1) An increase in the mean nuclear grain count to at least 6 grains/nucleus in excess of the concurrent negative control value, and/or
>
> 2) The percent of nuclei with 6 or more grains to increase above 10% of the examined population, in excess of the concurrent negative control, and/or
>
> 3) The percent of nuclei with 20 or more grains to reach or exceed 2% of the examined population.
>
> A dose-related increase in UDS for at least two consecutive applied concentrations is also desirable to evaluate a test material as active in this assay...If an isolated increase occurs for a treatment far removed from the toxic doses, the UDS will be considered spurious.
>
> The test material is considered inactive in this assay if none of the above conditions are met and if the assay includes the maximum applied dose or other doses that are shown to be toxic by the survival measurements. If little or no toxicity is demonstrated by any of the applied doses and the test material remains soluble in the test medium, the assay may be considered inconclusive...

A. Materials (continued)

After cultures were established, the dexamethasone and serum were removed from the medium which the investigators designated WME.

B. Methods

1. Liver cell preparation: The liver of a male rat (see above) was perfused in situ for approximately 4 minutes with Hanks' balanced salts (Ca^{++} and Mg^{++} free), 0.05 mM ethyleneglycol-bis (beta-aminoethyl ether)-N,N tetra-acetic acid (EGTA), and HEPES buffer (pH 7.0). Then the liver was perfused for approximately 10 minutes with WME containing 70 to 100 Units collagenase per ml. The perfused liver was then excised, placed in a culture dish with WME and collagenase, and disrupted mechanically. The resulting tissue suspension was then filtered through sterile cheesecloth to remove debris and cell clumps. The filtrate was centrifuged, and the cell pellet was resuspended in WME supplemented with 5% fetal bovine serum and 2.4 uM dexamethasone.

The report stated that a series of 35-mm culture dishes containing 25 mm round coverslips were seeded with approximately 0.5 X 10^6 cells. Cultures were established by incubation at 37° C in a humidified atmosphere containing 5% CO_2 for 1.5 to 2 hours. At the end of the attachment period, cells that are not attached to the coverslips are removed, and the medium is replaced with WME.

2. Cytotoxicity assay: Established cultures are exposed to 15 concentrations of the test substance ranging from 1000 ug/ml to 0.025 ug/ml (2-fold dilutions). The test substance is added to the cultures within 3 hours of their preparation, and the cultures are incubated for 20-24 hours before a viable cell count is obtained by the trypan blue exclusion method. The report stated that at least 6 concentrations were chosen for the main assay. The highest dose resulting in a sufficient number of survivors with intact morphologies was used as the starting point for determination of the 6 concentrations to be tested.

3. UDS assay: The report stated that the UDS assays were started within three hours after the attachment period of test cultures. The test cultures were given fresh WME containing 1% fetal bovine serum, 1 uCi/ml ^3H-thymidine, and test substance at concentrations of 5, 10, 25, 50, 100, 250, 500, or 1000 ug/ml. There were five cultures for each test and control group, and two of those were used for cytotoxicity determinations. After incubation of these cultures for 18 to 19 hours, the monolayers were washed twice with WME to terminate the exposure to the test substance. The two cultures in each test group used to monitor cytotoxicity were incubated in WME for another 20 to 24 hours before viable cell counts were obtained using the tyrpan blue exclusion method mentioned above.

The three cultures used for the UDS assay were treated with 1% sodium citrate solution for 10 minutes to swell the nuclei of the labeled cells. The cells were then fixed in acetic acid:ethanol (1:3) and dried for 24 hours. The coverslips with the fixed and dried cultures were mounted to glass slides (cells up) and dipped into autoradiographic emulsion. The dried slides were stored in darkness at 4° C for 7 to 10 days before they

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Reviewed by: Roger Gardner N.G. 11-24-87
Section 6, Toxicology Branch (TS 769C)
Secondary Reviewer: Judith Hauswirth, Ph. D. Jun 10/28/87
Section 6, Toxicology Branch (TS 769C)

DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Unscheduled DNA Synthesis (Guideline §84-2)

MRID NUMBER: 40095503

TEST MATERIAL: Pure thiophanate methyl (99.8% active ingredient; lot no. CP-10B) was used.

SYNONYMS: Thiophanate methyl; TOPSIN® M; 1,2-bis(3-methoxycarbonil-2-thioureido) benzene.

STUDY NUMBER(S): 21191

SPONSOR: Nippon Soda Co., Ltd

TESTING FACILITY: Litton Bionetics, Inc.

TITLE OF REPORT: Evaluation of Pure Thiophanate-Methyl in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay

AUTHOR(S): Myhr, B. C.

REPORT ISSUED: October, 1981

DISCUSSION AND CONCLUSIONS: There were adequate data presented to indicate that concentrations of 5 to 1000 ug Thiophanate Methyl per ml did not induce unscheduled DNA synthesis in primary rat hepatocytes *in vitro*.

Core classification: Acceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: 2-Acetyl aminofluorine (2-AAF; 0.05 ug/ml) was used as the positive control substance.

2. Vehicle: Dimethyl sulfoxide (DMSO) was used as the vehicle for the test substance.

3. Test species: Primary hepatocytes were obtained from a male Fischer 344 rat (see below for methods).

4. Media: Cells were maintained in Williams' Medium E supplemented with 5% fetal bovine serum, 2 mM L-glutamine, 2.4 uM dexamethasone, 100 U/ml penicillin, 100 ug/ml streptomycin sulfate, and 150 ug/ml gentamicin.

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II. REPORTED RESULTS (continued)

According to the statistical analysis, the investigators noted that there was a significant difference in overall mutation frequency between experiments 1 and 2, but no statistically significant effects were found with respect to mutation rate and dose-response or time of observation in the study.

III. DISCUSSION

The report stated that historical data were used to support the criteria for interpreting results as positive, but no historical data were included with the report. However, the largest difference between the negative control groups for the two trials (see the control group values for the 96-hour observation in the presence of S-9 mix, Table 3 above) was approximately 5-fold suggesting that the criterion is appropriate.

II. REPORTED RESULTS (continued)

Table 3

Mutation rate (calculated according to the formula on page 4 above)

Concentration (ug/ml)	Without activation 48 hours	96 hours	168 hours	With activation 48 hours	96 hours	168 hours
			First trial			
0	3.38	1.82	2.01	2.12	1.20	2.41
6.25	6.29	1.00	1.07	2.31	1.60	3.02
12.5	4.98	2.36	1.07	5.52	2.38	3.13
25.0	1.80	2.13	1.29	1.09	3.22	4.31
50.0	0.94	1.55	4.55	2.15	1.43	4.80
100.0	4.38	1.22	1.72	1.44	1.46	5.21
EMS						
1.0 mM	4.20	9.14	49.33	---	---	---
5.0 mM	25.62	35.37	59.05	---	---	---
10.0 mM	27.55	257.65	323.55	---	---	---
DMN						
1.0 mM	---	---	---	3.52	12.06	192.92
5.0 mM	---	---	---	11.32	50.70	250.27
10.0 mM	---	---	---	10.62	355.00	591.04
			Second trial			
0	7.52	6.40	6.43	4.34	7.02	6.65
6.25	13.27	17.31	13.02	3.81	5.17	3.70
12.5	6.38	7.12	9.42	2.50	4.31	7.48
25.0	5.64	7.27	11.27	2.74	19.39	3.85
50.0	8.13	13.43	10.47	3.54	13.55	7.82
100.0	2.08	5.06	6.43	4.07	14.12	10.00
EMS						
1.0 mM	4.92	81.27	134.08	---	---	---
5.0 mM	5.23	298.93	559.55	---	---	---
10.0 mM	5.69	750.45	1433.79	---	---	---
DMN						
1.0 mM	---	---	---	9.49	186.36	303.35
5.0 mM	---	---	---	9.63	396.61	---*
10.0 mM	---	---	---	16.98	603.92	579.44

*The report stated that data were not available for this point.

By the criteria mentioned above, thiophanate methyl did not increase in a reproducible manner the mutation rate at any test point by 5-fold above the solvent control mutation rate. Under these conditions, the investigators did not consider thiophanate methyl to be mutagenic.

II. REPORTED RESULTS (continued)

C. Mutagenicity assays: The viability of cultures in both mutagenicity assays is summarized in Table 2, and Table 3 summarized the mutation rates observed in both assays.

Table 2

Viability of Chinese Hamster V79 cells exposed thiophanate methyl in the main mutagenicity assays

Concentration (ug/ml)	Without activation		With activation	
	Mean no. colonies/plate*	Percentage survival	Mean no. colonies/plate*	Percentage survival
First assay:				
0.0	175.0	100	166.7	100
6.25	135.0	78	149.7	90
12.5	138.0	79	149.3	90
25.0	143.3	82	127.3	76
50.0	121.7	70	139.3	83
100.0	93.7	54	155.0	93
Second assay:				
0.0	163.3	100	159.0	100
6.25	114.3	70	158.7	100
12.5	126.7	78	158.0	99
25.0	120.0	73	132.0	83
50.0	101.3	62	103.7	65
100.0	93.3	57	93.3	62

*Mean of three plates.

II. REPORTED RESULTS

A. Solubility: The report noted no maximum attainable concentration of thiophanate methyl in DMSO, and the investigators chose to use 10 mg/ml as the highest concentration in the cytotoxicity and mutagenicity assays.

B. Test substance cytotoxicity: Results reported for thiophanate methyl and the vehicle control group are summarized in Table 1.

Table 1

Cytotoxicity of the thiophanate methyl to Chinese Hamster V79 cells

Concentration (ug/ml)	Without activation		With activation	
	Mean no. colonies/plate*	Percentage survival	Mean no. colonies/plate*	Percentage survival
0.0	211.7	100	147.3	100
0.39	177.3	84	178.7	121
0.78	116.3	55	162.0	110
1.56	117.7	56	157.3	107
3.13	129.7	61	129.3	88
6.25	100.7	48	174.0	118
12.5	158.3	75	153.0	104
25.0	137.3	65	179.0	121
50.0	108.7	51	164.3	112
100.0	106.3	50	165.7	106

*Mean of three plates.

B. Methods (continued)

period, 6-thioguanine was added to these cultures (15 ug/ml), and these cultures were incubated for 14 days. At the end of that time, the plates are stained with Giemsa solution, and the colonies which represent HGPRT mutants are counted.

For determination of cloning efficiency at the first expression time, three 60 mm Petri dishes are inoculated with 200 cells for each concentration of test substance or reference mutagen. These cultures are incubated for 8 days before they are stained and counted.

Procedures followed at the second expression time (96 hours following treatment) were the same as those performed at 48 hours. Similar procedures were followed at the 168-hour expression time with the exception of the propagation of treated cultures.

Two independent mutation assays following these procedures were conducted.

3. Data analysis: According to the report the following information was used at each dose level to calculate the mutation rate:

M = Number of mutants grown in selective medium

N = Number of cells spread in selective medium (200,000 X 5 cultures)

C = Number of clones grown in non-selective medium

n = Number of cells spread in non-selective medium (200 X 3 culture)

Mutation rate for each test concentration was calculated as follows:

$$\text{Mutation rate} = \frac{(\text{Sum of M})}{(N)\ [(\text{Sum of C})/n]}$$

Analysis of Variance (ANOVA) was applied to mutation frequency data. The protocol noted that when necessary data were transformed by unspecified methods in order to satisfy assumptions of homogeneous variation or normal distribution

According to the report, a positive result is indicated when the mutation frequency is increased in a dose-related manner, and a treatment level causes a five-fold increase above the mutation rate of the control.

A. Materials (continued)

Treatment medium without S-9 mix consisted of 8.9 ml minimal medium, 1 ml Hepes (20 mM), and 0.1 ml of the appropriate test solution. Treatment medium with the S-9 mix consisted of 4.9 ml minimal medium 5 ml S-9 mix, and 0.1 ml of the appropriate test solution.

B. Methods

1. Cytotoxicity studies: The report stated that preliminary cytotoxicity studies with and without metabolic activation were conducted to provide a basis for dose selection in the mutagenicity assays. The range of doses tested in the preliminary assay was based on the solubility of the test substance in the DMSO vehicle. Since the thiophanate methyl was found to be soluble, it was prepared in the vehicle at a maximum concentration of 10 mg/ml. The report indicated that 50 ul of DMSO solutions was added to 4.95 ml of culture medium to yield a solvent concentration of 1% (v/v). The protocol stated that 1% was the maximum solvent concentration to be used, and the maximum resulting thiophanate methyl concentration tested was therefore 100 ug/ml.

   Based on these considerations, the concentrations of thiophanate methyl evaluated for cytotoxicity with and without S-9 mix were 0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 ug/ml. Cultures of approximately 2 X 10^6 cells were exposed to each concentration in F75 culture flasks. Treatment medium was removed after three hours, and the cells were washed, trypsinized, and counted. Six-cm petri dishes were then inoculated with approximately 200 cells and incubated for 7 days. After the incubation period, the plates were stained with Giemsa solution, and colonies were counted. The percentage survival for each dose was calculated from counts for the treatment and vehicle control cultures, and selection of the highest dose was made on the basis of the concentration that resulted in a survival of 20% according to the report.

2. Mutation assays: The report stated that approximately 2 X 10^6 cells were plated in a 75 cm^2 culture flask with 10 ml of the culture medium (described above). These flasks were incubated until the next day when the culture medium was removed and the treatment medium was added to the cultures. The cultures were then incubated for three hours in medium containing 0, 6.25, 12.5, 25, 50, or 100 ug thiophanate methyl per ml. Subsequently, treatment medium was removed, and the cells were washed with saline and trypsinized. Samples of these cultures were plated to determine viability, and the remainder of each culture was placed in culture medium and incubated for 48 hours (first expression time).

   At the first expression time each culture was trypsinized and resuspended in supplemental medium for counting. Approximately one-million cells were reseeded in culture medium to propagate the population of treated cells, and incubation was continued until the second expression period ended (96 hours after dosing).

   In order to determine mutation frequency, five subcultures from each treatment level were started with approximately 200,000 cells in 100 mm Petri dishes containing 10 ml supplemental medium. After a three-hour incubation

A. Materialx (continued)

were incubated overnight to permit attachment of the cells. Cultures were incubated at 37° C in air containing 5% CO_2 and 100% relative humidity.

4. Metabolic activation (S-9) mixture: Groups of 15 or 5 rats of unspecified sex and strain were induced with phenobarbital and betanaphthoflavone which were administered intraperitoneally for 5 consecutive days as follows:

Day 1	phenobarbital at 30 mg/kg
Day 2	phenobarbital at 60 mg/kg
Day 3	phenobarbital at 60 mg/kg betanaphthoflavone at 80 mg/kg
Day 4	phenobarbital at 60 mg/kg
Day 5	phenobarbital at 60 mg/kg

On the sixth day, the rats were sacrificed. The livers were removed and homogenized in chilled 0.15 M KCl solution. The homogenate was centrifuged at 9000 X g, and the supernatant (S-9 fraction) was decanted and stored at -80° C until needed. According to the report, the protein concentration of the first batch (from 15 rats) was 35 mg/ml and the aminopyrine demethylase activity was 1.35 uM formaldehyde/gm. liver/5 min. Those respective values for the second batch were 35.2 mg/ml and 1.83 uM/gm. liver/5 min. According to the report, the two batches of S-9 homogenate were evaluated in an Ames assay using Salmonella typhimurium strain TA100 and 2-aminoanthracene and benzo(a)pyrene which depend upon metabolic activation for their mutagenic activity. The investigators stated that the S-9 preparations gave acceptable results, but no further details were included in the report.

An S-9 mix was prepared, and it included the following ingredients:

S-9 homogenate	3.0 ml
$MgCl_2$ (50 mM) + KCl (330 mM)	1.0 ml
Glucose-6-phosphate (15 mg/ml minimal medium)	1.0 ml
NADP (15 mg/ml minimal medium)	1.0 ml
NADPH (15 mg/ml minimal medium)	1.0 ml
Minimal medium	1.0 ml
Hepes (20 mM)	2.0 ml

5. Media: Minimal medium included the following ingredients:

Dulbecco's MEM	100 ml
L-Glutamine (200 mM)	10 ml
Sodium bicarbonate (7.5%)	49 ml
Antibiotic stock	2 ml
Sterile water	839 ml

Antibiotic stock consisted of 1 ml streptomycin sulfate (200 mg/ml) and 1 ml potassium penicillin G (200,000 IU/ml).

Supplemental medium consisted of minimal medium (900 ml) and fetal calf serum (100 ml).

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DATA EVALUATION RECORD

STUDY TYPE: Mutagenicity - Point Mutation Assay in Chinese Hamster Lung Fibroblasts (Guideline §84-2)

MRID NUMBER: 40005504

TEST MATERIAL: Technical grade Thiophanate Methyl (96.36% purity; lot number TM-767) was used.

SYNONYMS: Thiophanate methyl; TOPSIN® M; 1,2-bis(3-methoxycarbonil-2-thioureido) benzene.

STUDY NUMBER(S): 063013-M-05184

SPONSOR: Penwalt Corp.

TESTING FACILITY: Life Science Research, Rome-Toxicology Centre

TITLE OF REPORT: Gene Mutation in Chinese Hamster V79 Cells - Thiophanate-Methyl Technical.

AUTHOR(S): Tippins, R. S.

REPORT ISSUED: September 28, 1984

CONCLUSIONS: No mutagenic activity was observed in Chinese Hamster V79 cells exposed to 6.25 to 100 ug thiophanate methyl per ml with and without metabolic activation. There was adequate information presented in the report to support the conclusions of the investigators.

Core classification: Acceptable

I. PROTOCOL

A. Materials

1. Reference mutagens: Ethylmethanesulfonate (EMS) and dimethylnitrosamine (DMN) were used without and with S9 mixture, respectively. The three concentrations of EMS and DMN used in the assays described below were reported to be 1, 5, and 10 mM.

2. Vehicles: Dimethylsulfoxide (DMSO) was used for the test substance and distilled water was used for the positive control substances.

3. Test species: The V79 line of the Chinese Hamster lung fibroblast was used. The cells were routinely maintained in Delbecco's MEM medium supplemented with 10% fetal calf serum. Before treatment 75-cm^2 culture flasks were inoculated with approximately 2 X 10^6 trypsinized cells. These cultures

APPENDIX

Data Evaluation Records for the Following Studies

Tippins, R. S. September 28, 1984 Gene Mutation in Chinese Hamster V79 Cells -- Thiophanate-Methyl Technical. Unpublished report no. 063013-M-05184 prepared by Life Science Research, Rome Toxicology Centre. Submitted by Penwalt Corp. MRID No. 40005504

Myhr, B. C. October, 1981. Evaluation of Pure Thiophanate-Methyl in the Primary Rat Hepatocyte Unscheduled DNA Synthesis Assay Unpublished report no. 21191. prepared by Litton Bionetics, Inc. Submitted by Pennwalt Corp. MRID No. 40095503

A. Previously Submitted Mutagenicity Data

In a previous review (see Memorandum dated January 16, 1987. From: R. Gardner, Toxicology Branch, Hazard Evaluation Division. To: Lois Rossi, Product Manager, Registration Division. Subject: Request for Tolerances Residues of Thiophanate Methyl on Grapes and Rice (Petition #6F2343) and Food Additive Tolerance on Raisins (Petition #6H5486) (EPA Reg. No. 4581322) Tox. Proj. Nos. 1319 and 1320. Tox. Chem. No. 375A.), the Toxicology Branch stated:

> Microbial assays showed that thiophanate methyl did not cause reverse mutations of the frame shift or base pair substitution type in Salmonella typhimurium with or without liver microsomal activation, and the fungicide did not show differential toxicity in repair deficient and proficient strains of Bacillus subtilis.
>
> Thiophanate methyl also failed to induce mitotic gene conversion in Saccharomyces cereviciae (yeast). This study was a screen for a large number of chemicals which were tested at only one dose and without a positive control substance. The study is therefore considered to be incomplete.
>
> Thiophanate methyl at intraperitoneal doses ranging from 8 to 500 mg/kg/day (5 consecutive doses) did not affect fertility or induce dominant lethal mutations in mice. The 400 and 500 mg/kg doses caused mortality. No positive control substance was included in this study.
>
> The dominant lethal and yeast assays are considered to be unacceptable since they are incomplete.

The Toxicology Branch recommended that additional mutagenicity studies, specifically those which evaluate thiophanate methyl's potential to cause chromosomal damage in mammals, be conducted.

B. New Mutagenicity Data

No mutagenic activity was observed in Chinese Hamster V79 cells exposed to 6.25 to 100 ug thiophanate methyl per ml with and without metabolic activation.

Concentrations of 5 to 1000 ug thiophanate methyl per ml did not induce unscheduled DNA synthesis in primary rat hepatocytes in vitro.

C. Discussion

Although the new studies corroborate in mammalian systems the results in S. typhimurium and B. subtilis, they are not assays that specifically evaluate potential effects of thiophanate methyl on chromosomal structure. Therefore, additional mutagenicity testing is still needed.